IFNy production after 4 days of culture at 0.0625 mM and 0.25 mM of butyrate, respectively. Assays using purified LP CD4 T cells demonstrated that butyrate directly decreased LP CD4 T cell activation, proliferation and cytokine production in response to TCR/CD28 stimulation. Studies on specific T helper subsets revealed that butyrate inhibited proliferation of Th17 cells at lower concentrations (IC50:0.147 mM) compared with Th1 (IC50:0.229 mM) and Th22 (IC50:0.258 mM) and Th non-IL-22/IL-17/IFNy producing (IC50:2.14 mM) subsets. In addition, it appeared there was a paradoxical increase of HIV-1 infection levels at lower concentrations of butyrate (0.125 mM). DISCUSSION/SIGNIFICANCE OF IMPACT: The addition of butyrate to activated LP CD4 T cells decreases TCR-mediated activation in a dose-dependent manner, and butyrate acts directly on purified LP CD4 T cell populations independent of other cell populations. Butyrate differentially inhibited the proliferation of Th17, Th1, and Th22 subsets, with Th17 cells being the most sensitive to butyrate but increased the infection levels of all T helper subsets at low concentrations. Further studies are needed to determine the mechanism of butyrate's actions on LP Th cells and the sensitivity of Th17 cells to the inhibitory effects of butyrate. These results could help direct targeted manipulation of the colonic microbiome of HIV-1 infected individuals to help resolve inflammation and limit the impact of the infection in the gut mucosa and systemically.

2349

The role of interleukin-23 in human melanoma

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OBJECTIVES/SPECIFIC AIMS: Interleukin-23 (IL-23) promotes differentiation of naïve T-cells into Th17 cells, which drive the pathogenesis of autoinflammatory conditions such as psoriasis. IL-23-neutralizing antibody therapies are now in use for treatment of psoriasis, with promising results. Studies in mice have shown that IL-23 plays a role in inhibiting the growth, progression, and metastasis of melanomas. Thus, therapeutic neutralization of IL-23 in patients may inadvertently increase their susceptibility to development of melanoma. In this study, we aim to characterize expression of IL-23 receptors (IL-23R) in human melanocytes and melanoma cells and tissue and to study the effect of IL-23 on growth, proliferation, and tumorigenicity of these cells. METHODS/STUDY POPULATION: IL-23R expression was characterized using immunofluorescence staining, Western blot, and flow cytometric analysis. Response of melanoma and melanocytes to recombinant IL-23 treatment will be studied through similar methods in addition to assays of cell proliferation and tumorigenicity. RESULTS/ANTICIPATED RESULTS: Preliminary immunofluorescence staining and flow cytometry results indicate that both human melanoma and primary melanocytes express IL-23 receptors. Western blot analysis showed that melanoma cell line A375 expressed nearly twice the amount of IL-23R versus normal melanocytes ($p < 0.05$). Based on previous studies, we anticipate that addition of recombinant IL-23 to cultures of melanoma will reduce proliferative potential, and we expect similar addition to normal melanocytes will increase DNA repair mechanisms. DISCUSSION/SIGNIFICANCE OF IMPACT: In showing that human melanocytes and melanoma cells express IL-23 receptors, and potentially showing the inhibitory effect of IL-23 in the development of melanocytic neoplasms, our findings imply that using IL-23 neutralizing therapies may increase risk of developing melanoma, especially in patients who are already susceptible. As such, these therapies must be used with great care in these patients.

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The plasma contact system and its role in common variable immunodeficiency (CVID): An explorative study

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OBJECTIVES/SPECIFIC AIMS: Assess the presence of contact activation at baseline in sera from common variable immunodeficiency (CVID) patients with and without inflammatory complications compared with healthy controls. METHODS/STUDY POPULATION: CVID patients were recruited in the outpatient setting and the measurement of cleaved plasma HK (cHK) levels was determined by Western blot analysis, under reducing conditions, with quantitation of total and cHK bands using an Odyssey imaging system (Licor). One-way ANOVA test for differences among the 3 studied groups will be applied. Biomarkers C3, C4, C1 inhibitor levels and hs-CRP were also measured. RESULTS/ANTICIPATED RESULTS: Participant enrollment continues and to date, 9 CVID patients were studied, 7 with and 2 without inflammatory complications. Repeated determinations of cleaved HK% (cHK%) revealed an average of 1.20% (range: 0.46%–2.66%) in CVID patients with inflammatory complications and those without complications averaged 1.07% (range: 0.79%–1.35%). Healthy controls had an average cHK of 1.15% (range: 0.60%–2.10%). DISCUSSION/SIGNIFICANCE OF IMPACT: Cleaved kininogen detected in the sera of CVID patients was found at similar levels compared with healthy controls (cHK < 5%). Findings suggest that systemic activation of the contact system might be absent in CVID, however, future considerations include developing detection methods for local tissue activation.

2356

The nasopharyngeal microbiome is perturbed and associated with increased clinical severity during acute respiratory viral infection

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OBJECTIVES/SPECIFIC AIMS: We sought to investigate the role of the host microbiome during severe, acute respiratory infection (ARI) to understand the drivers of both acute clinical pathogenesis. METHODS/STUDY POPULATION: Nasopharyngeal swabs comprised of mixed cell populations at the active site of infection were collected from 192 hospitalized pediatric patients with ARI. We combined comprehensive respiratory virus detection and virus genome sequencing with 16S rRNA gene sequencing to evaluate the microbial content of the airway during ARI. This data was coupled with 11 clinical parameters, which were compiled to create a clinical severity score. The microbiome profiles were assessed to determine if clinical severity of infection, and/or specific virus was associated with increased clinical severity. RESULTS/ANTICIPATED RESULTS: We identified 8 major microbiome profiles classified by dominant bacterial genus, Moraxella, Corynebacterium, Staphylococcus, Haemophilus, Streptococcus, Alloiococcus, Schlegelella, and Diverse. Increased clinical severity was significantly associated with microbiome profiles dominated by Haemophilus, Streptococcus, and Schlegelella, whereas Corynebacterium and Alloiococcus were more prevalent in children with less severe disease. Independent of the microbial community, more than 60% of patients with the highest clinical severity were infected with either respiratory syncytial virus or rhinovirus. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results indicate that individually and in combination, both virus and microbial composition may drive clinical severity during acute respiratory viral infections. It is still unclear how the complex interplay between virus, bacterial community, and the host response influence long-term respiratory impacts, such as the development of asthma. Nonetheless, during ARIs therapeutic interventions such as antibiotics and probiotics may be warranted in a subset of patients that are identified to have both a virus and microbiome profile that is associated with increased pathogenesis to limit both acute and long-term phenotypes.

2027

The role of lysyl oxidase in systemic sclerosis-associated lung fibrosis

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OBJECTIVES/SPECIFIC AIMS: Systemic sclerosis (SSc) is a connective tissue disease of unknown etiology characterized by progressive fibrosis of the skin and multiple visceral organs. Effective therapies for SSc are needed. Lysyl oxidase (LOX) is a copper-dependent amide oxidase that plays a critical role in the crosslinking of the extracellular matrix (ECM). In this study, we investigated the role of LOX in the pathophysiology of SSc. METHODS/STUDY POPULATION: LOX expression and protein levels were measured in lung tissues and primary fibroblasts from patients with SSc and healthy controls. The effects of recombinant LOX (rLOX) were measured in vitro in primary fibroblasts, ex vivo in human lung tissues and in vivo in mice given bleomycin in combination with rLOX. LOX levels and activity were evaluated in lung fibroblasts treated with an endostatin-derived peptide that ameliorates fibrosis

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and in mice treated with bleomycin in combination with the peptide. Further, to differentiate the crosslinking activity of LOX from other potential effects, primary human fibroblasts were cultured with rLOX in the presence of the inhibitor, beta-aminopropionitrile. The expression levels of ECM (collagen and fibronectin), pro-fibrotic factors (IL-6 and TGF-beta), and transcription factor (c-Fos) were examined by real-time PCR, ELISA, immunoblotting, or hydroxyproline assay. RESULTS/ANTICIPATED RESULTS: LOX mRNA was increased in lung tissues and matching fibroblasts of SSc patients. rLOX-induced ECM production in vitro and ex vivo in lung fibroblasts and in human lung tissues maintained in organ culture, respectively. Additionally, TGF-beta and bleomycin induced ECM production, LOX mRNA expression and activity. Endostatin peptide abrogated these effects. In vivo, rLOX synergistically exacerbated pulmonary fibrosis in bleomycin-treated mice. The inhibition of LOX catalytic activity by beta-aminopropionitrile failed to abrogate LOX-induced ECM production. LOX increased the production of IL-6. IL-6 neutralization blocked the effects of LOX. Further, LOX induced c-Fos expression and its nuclear localization. DISCUSSION/SIGNIFICANCE OF IMPACT: LOX expression and activity were increased with fibrosis in vitro, ex vivo, and in vivo. LOX induced fibrosis via increasing ECM, IL-6 and c-Fos translocation to the nucleus. These effects were independent of the crosslinking activity of LOX and mediated by IL-6. Our findings suggest that inhibition of LOX may be a viable option for the treatment of lung fibrosis. Further, the use of human lung in organ culture establishes the relevance of our findings to human disease.

2045

The role of TGFβ in driving early cystic fibrosis lung disease

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OBJECTIVES/SPECIFIC AIMS: Transforming growth factor-beta (TGFβ) is a genetic modifier of cystic fibrosis (CF) lung disease. TGFβ's pulmonary levels in young CF patients and its mechanism of action in CF are unknown. We examined TGFβ levels in children with CF and investigated responses of human airway epithelial cells (AECs) and mice to TGFβ. METHODS/STUDY POPULATION: TGFβ levels in bronchoalveolar lavage fluid from CF patients ($n = 15$) and non-CF control patients ($n = 21$) < 6 years old were determined by ELISA. CF mice and non-CF mice were intratracheally treated with an adenoviral TGFβ1 vector or PBS; lungs were collected for analysis at day 7. Human CF and non-CF AECs were treated with TGFβ or PBS for 24 hours then collected for analysis. RESULTS/ANTICIPATED RESULTS: Young CF patients had higher bronchoalveolar lavage fluid TGFβ than non-CF controls ($p = 0.03$). Mouse lungs exposed to TGFβ demonstrated inflammation, goblet cell hyperplasia, and decreased CFTR expression. CF mice had greater TGFβ-induced lung mechanics abnormalities than controls; both CF human AECs and CF mice showed higher TGFβ induced MAPK and PI3K signaling compared with controls. DISCUSSION/SIGNIFICANCE OF IMPACT: For the first time, we show increased TGFβ levels very early in CF. TGFβ drives CF lung abnormalities in mouse and human models; CF models are more sensitive to TGFβ's effects. Understanding the role of TGFβ in promoting CF lung disease is critical to developing patient specific treatments.

2090

TL1 team approach to osteosarcoma cell detection

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OBJECTIVES/SPECIFIC AIMS: The objective of our collaboration is to develop a strong transdisciplinary team consisting of microfluidics engineers, cancer biologists, and clinicians, to identify cell surface markers capable of detecting circulating osteosarcoma cells (COC) using microfluidic devices. Our goals are 3-fold: (1) Identify cell surface markers unique to osteosarcoma (OS) for COC isolation, (2) develop a Geometrically Enhanced Mixing (GEM) device to isolate COCs, and (3) Evaluate the efficacy of GEM device to detect COCs in OS patients under treatment. The long-term goal is to utilize this cell detection approach to correlate the presence of COC with metastatic incidence. METHODS/STUDY POPULATION: To identify a marker to capture COCs we are utilizing flow cytometry and microfluidic capture devices. Flow cytometry will be used to evaluate the relative expression of epithelial cell adhesion molecule (EpCAM), CD45, cell surface vimentin (CSV), insulin-like growth factor 2 (IGF2R), interleukin 11 receptor subunit alpha (IL-11Rα), ganglioside 2 (GD2), and receptor activator of nuclear factor κ-B (RANK) on a panel of OS cell lines. These cell surface markers were selected based on an extensive review of OS cell surface markers. OS cell capture efficacy will be assessed by passaging a known concentration of OS cells through a GEM microfluidic device coated with antibodies targeting the selected marker, as indicated by flow cytometry. Once captured, COCs on the device will be analyzed and the capture efficiency for the indicated marker will be measured. ANOVA will be used to determine any significant difference in capture efficiency between marker types. Once an optimal marker or panel of markers has been selected we will conduct capture studies using OS cell spiked blood samples followed by clinical samples obtained from OS patients. In clinical samples, COC detection will be validated using the FDA approved triple immunocytochemistry technical definition of a circulating tumor cell (CTC). This will enable COCs to be differentiated from the normal whole blood cell population by selecting for CD45 −, EpCAM +, and cytokeratin + cells. RESULTS/ANTICIPATED RESULTS: Our preliminary studies have shown that on our microfluidic device, EpCAM, a marker commonly used to identify circulating tumor cells in other cancer settings, has a poor capture efficiency (15.9% + 7.7%) for HU09 OS cells while the same setup with EpCAM has a capture efficiency of 56.9% + 2.7% for BXPc-3 pancreatic cells. We therefore anticipate our flow cytometry studies to show a low expression of EpCAM and CD45 for OS cell lines, while showing a moderate to high expression of CSV, IGF2R, IL-11Rα, GD2, and RANK. We expect to show a 60%–80% capture efficiency for markers selected for COC capture. Currently, CSV and GD2 are particularly promising as markers based on previously published studies. DISCUSSION/SIGNIFICANCE OF IMPACT: OS is the most common primary bone tumor and the third leading cause of pediatric cancer deaths. At diagnosis 80% of patients will present with metastasis, however only 20% of these cases are clinically detectable. Innovative strategies to identify patients at risk of metastasis would allow for stratification of intervention therapies. Currently, tumor recurrence and metastasis are primarily dependent on diagnostic-imaging modalities such as computerized tomography or positron emission tomography scans. Unfortunately, these imaging modalities can only detect tumor masses of significant size (106 tumor cells). Liquid biopsies are a novel alternative to current diagnostic imaging systems to monitor metastatic incidence and treatment efficacy. The detection of CTCs through routine blood sampling has the potential to be used clinically for earlier detection, monitoring the treatment of metastatic cancers and surveying the effect of therapeutic interventions on metastasis. To date, the majority of the studies on CTCs have evaluated their presence in carcinomas. Although sarcomas are rare, they generally have a poor prognosis. This study will address one of the unmet medical needs in the field of CTC detection; the identification of cell surface OS makers to improve binding specificity, increase purity, and maintain a high capture efficiency. This phase of our proposal will evaluate the most abundant and conserved markers across a panel of OS cell lines. Once a marker or panel of markers is selected, we will begin to develop a microfluidic device that can be used clinically to detect CTCs in this disease setting.

2303

Trauma-related acute respiratory distress syndrome (ARDS) in India: Current incidence and management strategies

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OBJECTIVES/SPECIFIC AIMS: Aim 1: To determine the true incidence of trauma-related acute respiratory distress syndrome (ARDS) in India. We propose to perform a prospective observational study to determine the incidence of ARDS in India. Aim 2: To perform a preliminary assessment of risk factors for ARDS in the Indian trauma population. We will leverage these findings against the global ARDS data to provide a foundation for further interventional studies. Aim 3: To evaluate the current management strategies and patient outcomes from ARDS in trauma subjects admitted to the Jai Prakash Narayan Apex Trauma Center (JPNATC). These findings will identify areas in need of practice-based performance improvement in ARDS therapies in India. METHODS/STUDY POPULATION: This application proposes an observational study of trauma patients with ARDS, a population that continues to have substantial in-hospital mortality. The approximate number of ICU-admitted trauma cases for the study period is 1700. Specific data elements to be collected include patient demographics, comorbidities, mechanism of injury, Injury Severity Score, risk factors for ARDS, sequential organ failure and assessment scores, vital signs, laboratory values, and evidence-based treatments received, including mechanical ventilation and adjunctive therapies. Outcome data will include discharge location, ICU and hospital length of stay and

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